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Yogita C. Bannore[1]
Kelly D. Chenault[2]
Hassan A. Melouk[3]
Ziad El Rassi[1]

[1]Department of Chemistry, Oklahoma State University, Stillwater, OK, USA
[2]USDA-ARS, Wheat, Peanut, and Other Field Crops Unit, Stillwater, OK, USA
[3]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK, USA

Original Paper

Capillary electrophoresis of some free fatty acids using partially aqueous electrolyte systems and indirect UV detection. Application to the analysis of oleic and linoleic acids in peanut breeding lines

This study has shown for the first time the suitability of CE with a partially aqueous electrolyte system for the analysis of free fatty acids (FFAs) in small portions of single peanut seeds. The partially aqueous electrolyte system consisted of 40 mM Tris, 2.5 mM adenosine-5′-monophosphate (AMP) and 7 mM α-CD in (*N*-methylformamide) NMF/dioxane/water (5:3:2 by volume) mixture, pH 8 – 9. While AMP served as the background UV absorber for indirect UV detection of the FFAs, the α-CD functioned as the selectivity modulator by affecting the relative effective electrophoretic mobilities of the various FFAs due to their differential association with α-CD. This CE method allowed the screening of peanut seeds for their content of oleic and linoleic acids, which is essential in breeding of peanuts of high-oleic acid content. The extraction method of FFAs from peanut seeds is very reproducible with a high recovery approaching quantitative yield (∼97% recovery).

Keywords: Capillary electrophoresis / Fatty acids / Linoleic acid / Oleic acid / Peanut breeding lines

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1 Introduction

Fatty acids are an important class of organic compounds known as “lipids”. Fatty acids encountered in plants and animals are the carboxylic acids often having a long, unbranched aliphatic carbon chain, which can be saturated or unsaturated [1]. In nature, fatty acids are bound to other molecules forming triglycerides and the breakdown of these triglycerides yields free fatty acids (FFAs) and glycerol.

Due to their importance in the food industry, the analysis of fatty acids in food matrices plays a key role in controlling product stability, quality, and chemical properties of edible oils and fats. In this study, peanut (*Arachis hypogaea* L.) oil was evaluated for its fatty acids composition, *e.g.*, oleic acid and linoleic acid contents. Peanut oil is very common in daily household cooking because of its high smoke point relative to other cooking oils. Also, peanut seeds hold fourth rank in world production [2]. Peanuts are composed principally of oils (44 – 56%) and proteins (22 – 30%) [3, 4]. The oil consists mainly of unsaturated fatty acids making it more susceptible to oxidation. The stability and nutritional quality of the oil depends on the relative proportion of saturated and unsaturated fatty acids. Palmitic, oleic, and linoleic acids comprise approximately 90% of the total fatty acid composition along with some other fatty acids [5, 6]. In this work, the quantitative determination of oleic acid and linoleic acid content of peanut oils was of major interest. Peanuts with normal oleic acid content go rancid on the shelf in about a year. High-oleic acid content along with low linoleic acid content is beneficial in increasing product shelf life [7], product flavor, decreasing rancidity, and also offers consumers health advantage by reducing the blood level of LDL cholesterol [8, 9]. Peanut seeds are classified as high oleic if the oleic acid content is 70% or greater while they fall under normal oleic acid category if the oleic acid content is less than 70% [10].

To produce peanut oils with high-oleic acid content, peanut breeding is carried out where genetic manipulation is employed to develop peanut lines with elevated oleic acid content [11]. In early generations of peanut

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Correspondence: Professor Ziad El Rassi, Department of Chemistry, Oklahoma State University, Stillwater, OK 74078-3071, USA
E-mail: elrassi@okstate.edu
Fax: +1-405-744-1235

Abbreviations: AMP, adenosine 5′-monophosphate; **DMe-β-CD,** Dimethyl-β-CD; **FFA,** free fatty acid; **IS,** internal standard; **NMF,** *N*-methylformamide; **SDBS,** sodium dodecyl benzenesulfonate lines, the range of oleic acid can vary to some extent. Many early generations of peanut lines will produce mid-oleic acid seeds. These seeds will then produce plants that eventually result in high-oleic seeds after self-pollination over generations. Screening for those individual peanut seeds which are high in oleic acid content is an important part of breeding programs.

To screen for the fatty acids content of early generation seeds, a small portion (0.2 – 0.3 g) of the peanut seed is usually cut for FFA's analysis, keeping the rest of the seed intact for germination and subsequent plant generation. Therefore, there is a need for an analytical technique that is ideal for analyzing the small amount of peanut oil derived from a small portion of a single peanut seed. CE with its small sample requirement offers the ideal analytical platform for small amounts of peanut oils. In addition, CE is a versatile separation technique that is increasingly being employed in the analysis of complex natural matrices, *e.g.*, food (for a review see ref. [12]) and systems biology [13] due to the fact that CE offers high separation efficiency and unique selectivity.

Despite the fact that CE has been employed in the analysis of a wide variety of compounds, it has been rarely applied to peanut oil fatty acids investigations. The most traditionally used methods for the determination of fatty acids in natural samples including peanut oils have been GC [14, 15] and HPLC [16, 17], with HPLC methods offering slightly better precision than the GC-flame ionization detector methods [18]. However, both techniques require time-consuming derivatization steps of fatty acids to improve volatility in GC and increase detectability in HPLC.

One of the major concerns in analyzing FFAs by CE has been their limited solubility in aqueous electrolyte systems. This problem has been overcome by introducing nonaqueous CE (NACE) more than a decade ago [19] (for a review see ref. [20]). NACE as well as partially aqueous CE (*i.e.*, organic-rich CE) have found use in the separation of saturated and unsaturated FFAs. *N*-Methylformamide (NMF) – dioxane-based electrolyte systems [21, 22], and 100% methanol with 12.5 mM tetraethylammonium chloride [23] were effective in the separation of some long-chained fatty acids (≥C16). Also, effective in the separation of fatty acids have been some partially aqueous electrolyte systems containing 60% ACN [24], 60% methanol [25], 50% methanol [26], mixture of 10 mM sodium dodecyl benzenesulfonate (SDBS), 50% ACN, and Brij [27], SDS micelles with 20% methanol [28], mixture of 40% ACN and 30% ethanol [29], or mixture of 4 mM SDBS, 10 mM Brij 35, 2% 1-octanol, and 45% ACN [30].

Fatty acids do not possess strong chromophores in their structures, which prohibits their sensitive detection in direct photometric detection. A solution to this problem was provided by the introduction of indirect UV and indirect fluorescence detection in CE well over a decade ago for the sensitive detection of compounds that lack chromophores and fluorophores (for a review see ref. [31]). Among the various UV detectable coions (*i.e.*, background UV absorber) that proved useful in the indirect UV detection of FFAs are anthraquinone-2-caroxylic acid [21], adenosine-5′-monophosphate (AMP) [22], 3,5-dinitrobenzoic acid or trinitrobenzenesulfonic acid [24], *p*-anisate [25, 26], and SDBS surfactant [27, 30]. Indirect LIF detection of FFAs was carried out with the fluorescing dye coion merocyanine 540 [29].

In this report, the CE behaviors of five standard FFAs, which are common in most oils, namely palmitic, stearic, oleic, linoleic, and linolenic acids were first investigated in CE under various nonaqueous and partially aqueous electrolyte systems in order to achieve a baseline separation for these FFAs. The optimal electrolyte system was applied to the determination of oleic acid and linoleic acid in peanut oils in order to provide a CE method for the screening of high-oleic peanut seeds that are essentials in breeding programs.

2 Experimental

2.1 Instrumentation

The CE analysis of all standards and peanut samples was performed on a P/ACE MDQ (Beckman Instruments, Fullerton, CA, USA) equipped with a photodiode array detector and a 0 – 30 kV high-voltage power supply. The data were collected on an IBM PC configured with P/ACE MDQ gold software version 1.5. The capillary columns used for separation were untreated fused-silica capillaries with 50 μm id and 363 – 359 μm od from Polymicro Technologies (Phoenix, AZ, USA). The total and effective lengths were 60.2 and 50 cm, respectively. The experiments were performed at a constant voltage of 28 kV and the temperature was maintained at 20°C. All standards and samples were injected hydrodynamically for 3 s by the application of a pressure of 0.5 psi. The indirect UV detection was carried out at a wavelength of 254 nm using anthraquinone-2-carboxylic acid, *p*-anisate, or AMP as the background UV absorbers.

Refluxing of oil extracts was done in a Thermolyne dri-bath, *i.e.*, heating block (Dubuque, IA, USA). The samples were centrifuged in a 1550-RPM Centricone (Precision Scientific, Chicago, IL, USA) and sonicated in a Branson Ultrasonic Cleaner (Branson Ultrasonic, Danbury, CT, USA). The other equipments used were Speed Vac (model-SC110) equipped with a Refrigerated Vapor Trap (Model-RVT4104) along with vacuum pump components Model-VLP120 (Savant Instruments, Holbrook, NY, USA) and a pH meter from Jenco electronics (San Diego, CA, USA).

2.2 Materials and reagents

Saturated FFAs standards such as stearic acid (C18:0), palmitic acid (C16:0), and nonadecanoic acid (C19:0), and unsaturated FFAs such as oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), AMP monohydrate from yeast, Tris, α-CD, and β-CD were obtained from Sigma (St. Louis, MO, USA). Dioxane, HCl, diethyl ether, and anhydrous sodium sulfate were from Fischer Scientific (Fairlawn, NJ, USA) and NMF, anthraquione-2-carboxylic acid and 4-methoxybenzoic acid were from Aldrich (St. Louis, MO, USA). Dimethyl-β-CD (DMe-β-CD) was obtained from Fluka (Fluka Chemie, Buchs, Switzerland), hexane from EM Science (Cherry Hill, NJ, USA), and KOH was from Mallinckrodt (Paris, Kentucky, USA). Ethanol and methanol were purchased from AAPER Alcohol and Chemical (Shelbyville, KY, USA).

2.3 Preparation of solutions

Three different electrolyte systems were evaluated in this study. The first nonaqueous electrolyte system (*i.e.*, running electrolyte) that was tried consisted of 40 mM Tris and 2.5 mM anthraquinone-2-carboxylic acid in NMF/dioxane (3:1 v/v) [21]. The various FFAs standard stock solutions were prepared using neat NMF as the solvent to give a concentration of 5 mM. All standard solutions used in the CE experiments were prepared by diluting an aliquot of the stock solutions in the running electrolyte.

The second electrolyte system was hydro-organic system of water/methanol (1:1 v/v) containing 10 mM Tris, 5 mM *p*-anisate, and 1 mM DMe-β-CD [26]. Tris was partially neutralized with *p*-anisic (4-methoxybenzoic) acid to pH 8.1. The stock solutions of the various standard FFAs were prepared in neat methanol.

The third running electrolyte system in its final and optimal composition consisted of 40 mM Tris, 2.5 mM AMP and 7 mM of α-CD in a mixture of NMF/dioxane/water (5:3:2 by volume). The stock solutions of all standard FFAs were prepared by dissolving appropriate amounts of acids in NMF/dioxane (4:1 v/v) to give concentration of 5 mM. All standard solutions were prepared by diluting an aliquot of the stock solutions in the running electrolyte.

The concentrations of standard solutions for the two FFAs oleic acid (C18:1) and linoleic acid (C18:2) used for calibration were 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 mM, while the FFA C19:0 used as the internal standard (IS) had a concentration of 0.5 mM. The IS was first dissolved in dioxane, and then NMF was added. The IS was added after the extraction. Each concentration of the calibration curve was repeated thrice. All stock and standard solutions were stored in the refrigerator.

2.4 Electrophoretic conditions

On a daily basis and before the start of the experiments, the separation capillary was flushed successively with the following solutions and durations: 1 M NaOH for 10 min, followed by water for 3 min, 0.1 M HCl for 10 min, water again for 3 min, and finally with the running electrolyte for 5 min using the PÅCE MDQ instrument setting and applying a pressure of 65 psi to the vial for each washing step. The successive washing was followed by equilibration of the separation capillary with freshly prepared running electrolyte solution for 20 – 30 min at the running voltage (*i.e.*, 28 kV).

Hydrodynamic injection of all standards and samples was done for 3 s at 0.5 psi (1 psi = 6895 Pa) at the anodic end. The capillary was pressure rinsed with the running electrolyte for 2 min at 65 psi between successive injections. Each run was performed at 28 kV with the capillary and sample temperature maintained at 20°C, and the detection was set at 254 nm. The running electrolyte in the inlet reservoir was changed several times a day while the outlet reservoir electrolyte was changed daily. During weekends and at nights, the capillaries were stored in water.

2.5 Peanut breeding

The analysis of fatty acid was carried out primarily on two peanut lines (Okrun and ARSOK-R1) and to a lesser extent on some other lines. Okrun is a peanut cultivar that was released in 1980s [32] while ARSOK-R1 is an advanced breeding line under development for variety release [33]. Okrun, classified as the runner variety, results from the cross of Florunner and Spanhoma [32]. Both lines, Okrun and ARSOK-R1, were planted in the test plots of the Oklahoma State University Agricultural Experiment Station in Ft. Cobb, Oklahoma around May 15, 2006 and harvested around October 15, 2006. To harvest, the plants were first dug (uprooted and turned over) and then they were left in the windrows for 3 days, which is sufficient, for the peanut hay to dry. After 3 days, the hay is dry enough to be separated from the pods (thrashed). The pods are placed in a peanut dryer which forces warm air through a trailer and dries the pod. Finally, the pods are shelled and the seeds are stored at 4°C until analysis.

2.6 Oil and fatty acids extraction from single peanut seeds

The seeds used for the determination of FFAs were sound and mature. Prior to analysis, the seeds were brought to room temperature. After removing the seed coat, a small portion of the seed was cut from the distal end (away from the embryo), weighed and ground to paste in a mor-

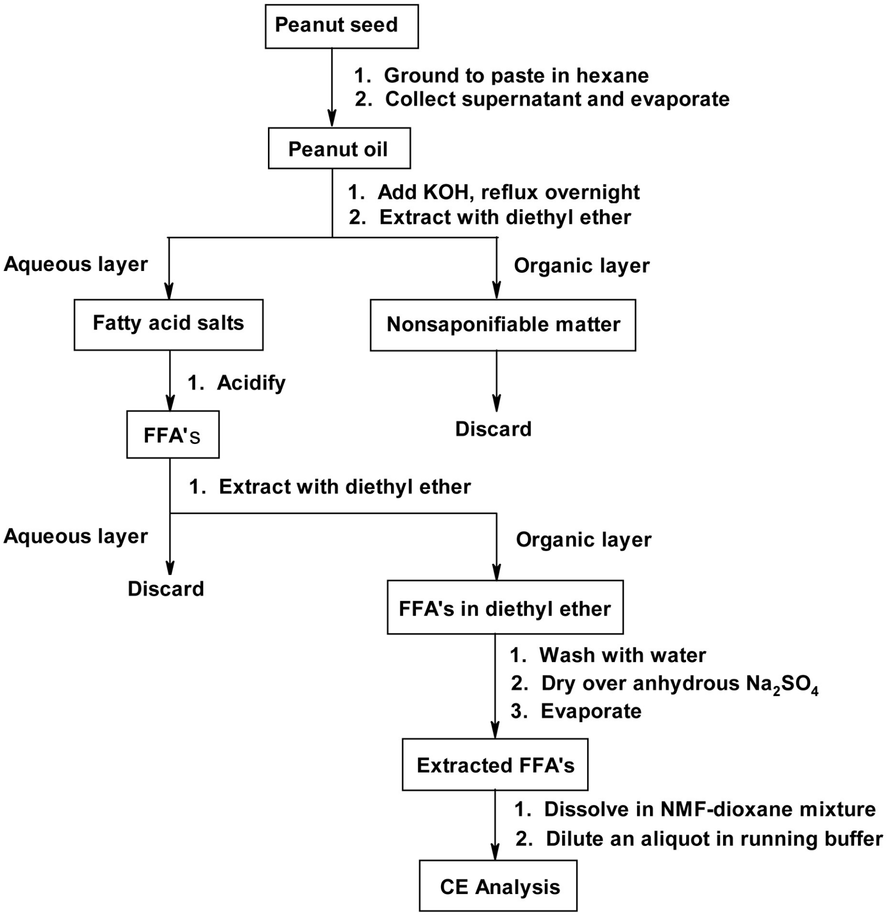
Peanut seed
1. Ground to paste in hexane
2. Collect supernatant and evaporate
Peanut oil
1. Add KOH, reflux overnight
2. Extract with diethyl ether
Aqueous layer
Organic layer
Fatty acid salts
Nonsaponifiable matter
1. Acidify
FFA's
Discard
1. Extract with diethyl ether
Aqueous layer
Organic layer
Discard
FFA's in diethyl ether
1. Wash with water
2. Dry over anhydrous Na_2SO_4
3. Evaporate
Extracted FFA's
1. Dissolve in NMF-dioxane mixture
2. Dilute an aliquot in running buffer
CE Analysis

Figure 1. Chart showing the various steps for oil and free fatty acids extraction from a portion of a single peanut seed.

tar-pestle in a sufficient volume of hexane. The slurry was transferred into a vial. The remaining paste was scraped and washed into the vial. The mortar was washed two to three times with hexane as required for complete transfer of the contents. The vial was vortexed for *ca.* 1 min and centrifuged for about 20–25 min at 10 000 rpm. The supernatant layer was collected and the hexane was evaporated using speed vacuum. The peanut oil left behind was further used for the extraction of FFAs.

The extraction of peanut lines was accomplished using the method of Dermaux *et al.* [34] with some modifications. To 20–25 mg of oil, 0.55 mL of 1 M potassium hydroxide in 95% ethanol solution was added and subjected to overnight reflux in a dri-bath. Thereafter, the mixture was cooled to room temperature, and then transferred to a separatory funnel by rinsing the reflux vial with 1.5 mL water. The nonsaponifiable matter was extracted using 1.5 mL of diethyl ether. The aqueous layer containing fatty acid salts was separated from the organic layer and then acidified to pH 2.0 with 1 M HCl. The FFAs formed were extracted with 6 × 1 mL diethyl ether. All organic layers were collected, washed with 1 mL of water, and dried over anhydrous sodium sulfate. The solvent was evaporated at room temperature in the fume hood. The extraction procedure is summarized in Fig. 1.

2.7 Sampling of extracted fatty acids from peanut oil for CE analysis

For direct CE analysis, the extracted FFAs were dissolved in 0.5 mL of NMF/dioxane (1:1 v/v) mixture. This was done by first dissolving the FFAs in dioxane followed by the addition of NMF and then vortexing for 10–15 s. An aliquot (1 or 2 μL) from this solution was taken and diluted (50-fold or 100-fold) in the final running buffer and vortexed for 4–5 s. The sample was then pressure injected in the CE instrument.

3 Results and discussion

3.1 CE of fatty acids

Because of their weak carboxylic acid groups, free fatty acids ionize readily in basic solutions forming FFA anions, which favor their differential migration in CE. One important factor that should be considered during

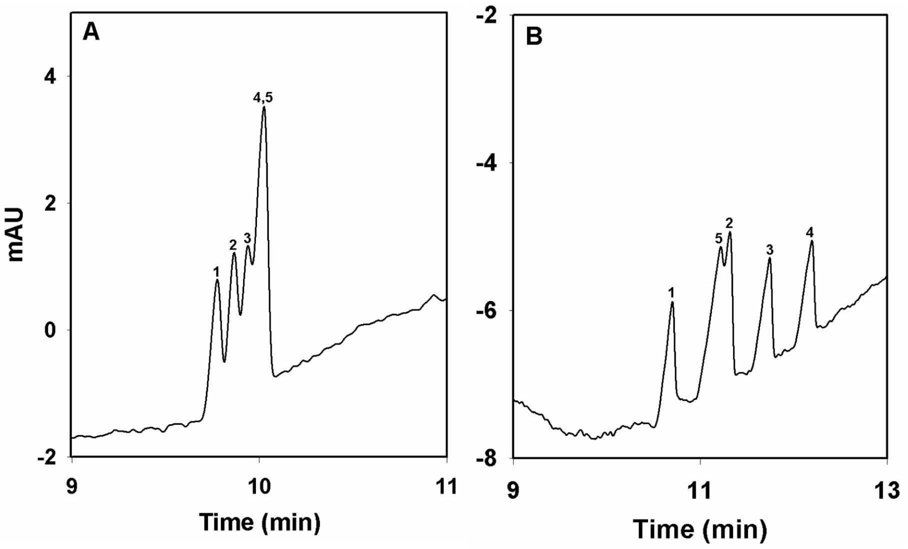

Figure 2. Electropherograms of five standard FFAs using in (A) a nonaqueous electrolyte system consisting of 2.5 mM anthraquinone-2-carboxylic acid (UV absorber) and 40 mM Tris in NMF/dioxane (3:1 v/v), pH 10–11, and in (B) a partially aqueous electrolyte system consisting of 10 mM Tris, 5 mM *p*-anisate and 1 mM DMe-β-CD in a water/methanol (1:1 v/v) mixture whose pH was adjusted to 8.1. Hydrodynamic injection for 3 s at 0.5 psi, applied voltage of 28 kV, and detection at 254 nm. Peak identification: 1, C18:0; 2, C18:1; 3, C18:2; 4, C18:3; 5, C16:0.

FFAs separation in CE especially in aqueous media is their solubility, which tends to decrease as the hydrophobic alkyl chain of the fatty acid increases. This fact has led to developing and investigating nonaqueous CE systems. Furthermore, FFAs lack chromophoric centers that would allow their sensitive detection in the UV, thus the decision to perform indirect UV detection.

The first electrolyte system that was examined in the present CE study consisted of a nonaqueous medium, which was described by Drange and Lundanes [21]. It was composed of 2.5 mM anthraquinone-2-carboxylic acid and 40 mM Tris in NMF/dioxane (3:1 v/v). Anthraquinone-2-carboxylic acid was the background UV absorber in the running electrolyte having UV maximum at 263 nm. The FFAs were detected by indirect UV detection at the wavelength of 254 nm. A typical electropherogram is shown in Fig. 2A, and the CE run was achieved in slightly more than 10 min. This was facilitated by a relatively fast EOF with a migration time t_0 of ~6.6 min. This may be attributed to the high dielectric constant to viscosity ratio of the NMF [21]. As shown in Fig. 2, this nonaqueous electrolyte system yielded poor resolution between the FFAs investigated. The palmitic acid (C16:0) and the linolenic acid (C18:3) formed the critical pair, which was not resolved.

The second electrolyte system investigated in this report consisted of a partially aqueous medium containing 10 mM Tris, 5 mM *p*-anisate, and 1 mM DMe-β-CD in a binary solvent mixture of water/methanol (1:1 v/v). This electrolyte system, which was first described by Collet and Gareil [26], had the *p*-anisic acid as the background UV absorber while DMe-β-CD was introduced to adjust the selectivity, which is related to the difference in effective mobilities. The standard FFAs analyzed in this hydro-organic solvent mixture are shown in Fig. 2B where palmitic acid (C16:0) was not well resolved from oleic acid (C18:1). In addition, baseline instability and peak fronting were observed and problems regarding reproducibility were also encountered, and therefore the system was not further pursued.

The next electrolyte system under study was described by Haddadian *et al.* [22], and consisted of 40 mM Tris and 2.5 mM AMP in NMF/dioxane/water (5:4:1 by volume) mixture. The standard FFAs were detected by indirect UV detection at 254 nm. The elution order of the FFAs was the same as in the nonaqueous NMF/dioxane system (see above), and the palmitic acid (C16:0) coeluted with linolenic acid (C18:3). The resolution in this partially aqueous system is superior to that in the neat NMF/dioxane system, compare Fig. 3A to Fig. 2A. This observation revealed the importance of adding water to the electrolyte system in terms of improved FFAs resolution. The effect of the water content of the running electrolyte on resolution was studied by varying its percentage in the final running electrolyte. At 15% water (Fig. 3B), there was a slight improvement, which was further enhanced by adding 20% of water (Fig. 3C) to the nonaqueous mixture. At 30% water (Fig. 3D), no significant effect was observed and thus, 20% was considered as the best water content in NMF/dioxane/water mixture. Increasing the water content in the running electrolyte was done at the expense of decreasing the percentage of dioxane while keeping the NMF content constant. Decreasing the percent of NMF instead of decreasing the percent dioxane would have been detrimental to EOF [22], which in turn would have increased the analysis time.

However, adjusting the percentage of water did not lead to separating palmitic acid from linolenic acid. To improve the selectivity between these two coeluting acids, a selectivity modulator that affects the effective mobilities of the two acids was needed. In this regard, 0.2% w/v Brij 35 was added to the running electrolyte

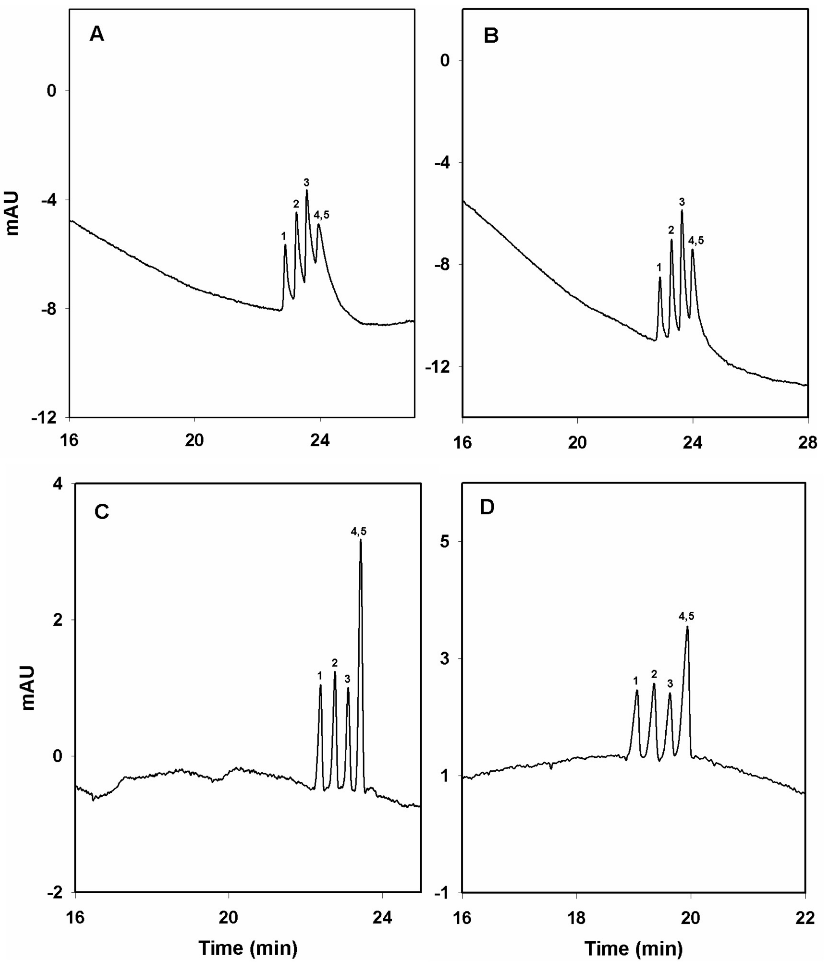

Figure 3. Effect of water on FFAs resolution. Electropherograms of five standard FFAs using partially aqueous electrolyte consisting of 40 mM Tris and 2.5 mM AMP in (A) NMF/dioxane/water (5:4:1 by volume), (B) NMF/dioxane/water (5:3.5:1.5 by volume), (C) NMF/dioxane/water (5:3:2 by volume), and (D) NMF/dioxane/water (5:2:3 by volume) mixture, pH in the range of 8–9. Hydrodynamic injection for 3 s at 0.5 psi, applied voltage of 28 kV, and detection at 254 nm. Peak identification: 1, C18:0; 2, C18:1; 3, C18:2; 4, C18:3; 5, C16:0.

consisting of 40 mM Tris and 2.5 mM AMP in NMF/dioxane/water (5:3.5:1.5 by volume) mixture. Although the nonionic surfactant Brij 35 would enhance the solubility of FFAs in the running electrolyte, its presence as a hydrophobic selector that would bind to the various FFAs and modulate their migration did not resolve the coeluting pair, palmitic acid and linolenic acid.

Next, CDs were incorporated into the running electrolyte to modify FFAs electromigration and in turn selectivity. In fact, CDs have been known to resolve difficult to separate solute pairs [35, 36]. In the case of FFAs presented here, α-CD was more effective than β-CD in modifying the selectivity under otherwise identical running conditions; compare Fig. 4B to 4A. On this basis, the electrolyte system incorporating α-CD was further investigated and different concentrations of α-CD were examined. As mentioned above, in the absence of α-CD, the palmitic acid (C16:0) comigrated with linolenic acid (C18:3) whereas between 2 and 4 mM α-CD, it coeluted with linoleic acid (C18:2) indicating that the palmitic acid formed a strong host–guest interaction with α-CD. Palmitic acid partially coeluted with linoleic acid (C18:3) at 5 mM concentration of α-CD and complete resolution was observed in the range of 6–7 mM α-CD. Above 8 mM concentration of α-CD, the mobility of palmitic acid showed extreme reduction and was almost equal to the mobility of the oleic acid (C18:1). The 7 mM concentration of α-CD was used for the analysis of FFAs in the peanut oil, see next section.

The effects of the concentrations of α-CD on the electroosmotic mobility, apparent mobility, and effective electrophoretic mobility are shown in Fig. 5. As can be seen in Fig. 5A, the effective electrophoretic mobility of the FFAs decreased with increase in α-CD due to the decrease in the charge-to-mass ratio of the FFA^- ion upon its binding to α-CD to produce the complex $CDFFA^-$. Thus, the electrophoretic mobility of $CDFFA^-$ is less than that of FFA^-. That is $\mu_{ep,CDFFA^-} < \mu_{ep,FFA^-}$. The effective electrophoretic mobility (μ_{eff}) of FFA^- is given by the following equation:

$$\mu_{eff} = \mu_{ep,FFA^-}\chi_{FFA^-} + \mu_{ep,CDFFA^-}\chi_{CDFFA^-} \qquad (1)$$

where μ_{ep,FFA^-} is the electrophoretic mobility of the free acid, $\mu_{ep,CDFFA^-}$ the electrophoretic mobility of the complex $CDFFA^-$, χ_{FFA^-} the mole fraction of the free acid, and χ_{CDFFA^-} is the mole fraction of the complex $CDFFA^-$ given

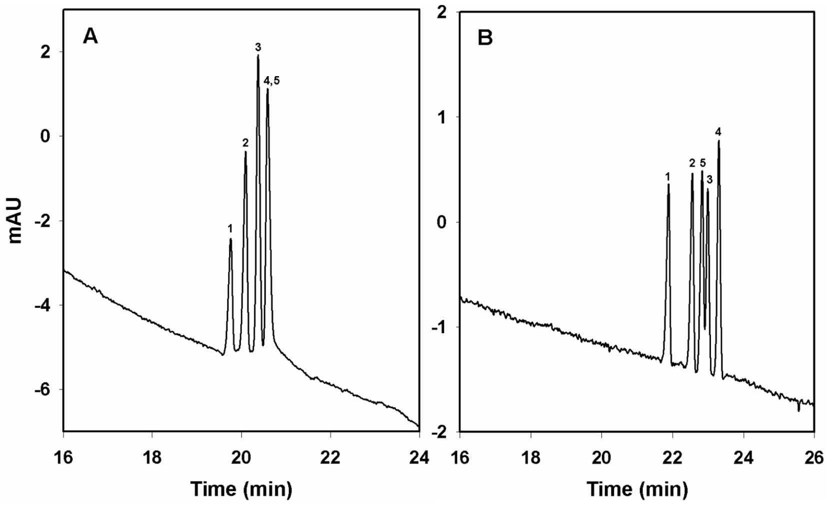

Figure 4. Electropherograms of five standard FFAs in presence of (A) 5 mM β-CD and (B) 5 mM α-CD in the partially aqueous electrolyte consisting of 40 mM Tris and 2.5 mM AMP in NMF/dioxane/water (5:3:2 by volume) mixture, pH between 8 and 9. Hydrodynamic injection for 3 s at 0.5 psi, applied voltage of 28 kV, and detection at 254 nm. Peak identification: 1, C18:0; 2, C18:1; 3, C18:2; 4, C18:3; 5, C16:0.

by $\chi_{\mathrm{CDFFA}^-} = 1 - \chi_{\mathrm{FFA}^-}$. On this basis, it is clear that as the amount of added α-CD is increased the mole fraction χ_{CDFFA^-} is increased while that of the FFA⁻ is decreased. The net result is a decrease in the effective electrophoretic mobility of the solute. This behavior is seen in Fig. 5A by the monotonous decrease in the effective electrophoretic mobility in the concentration range studied.

Since the decrease in the effective electrophoretic mobility is continuous in the concentration range of α-CD studied, the apparent mobility of the FFAs almost paralleled that of the EOF upon varying α-CD concentration (compare Figs. 5B and C). In general, the EOF increased first when going from 0 to 2 mM α-CD, and then decreased slightly thereafter in the range between 2 and 10 mM α-CD (Fig. 5B). This behavior of EOF may be explained by the fact that the addition of α-CD to the running electrolyte would increase the dielectric constant of the medium, which would lead to an abrupt increase in the EOF. However, as the α-CD concentration is further increased the viscosity of the medium is increased and also a thicker layer of α-CD is then bound to the capillary wall, which would lead to an increase in the local viscosity at the wall where the EOF usually develops [37]. The following equation relates the zeta potential ζ in the electric double layer (EDL) and EOF supports the above explanation for the change in EOF upon varying α-CD concentration in the running electrolyte [37, 38]:

$$\mu_{\mathrm{eo}} = \varepsilon\eta\zeta \approx \varepsilon \int_0^{\zeta} \frac{1}{\eta} \mathrm{d}\Psi \qquad (2)$$

where μ_{eo} is the electroosmotic mobility, ε the medium dielectric constant, η the viscosity of the medium, and Ψ is the electric potential. Hjerten [37] through the use of Eq. (2) illustrated that the EOF mobility will go to zero as the viscosity of the buffer inside the EDL, due to adsorption of polymer, approaches infinity even though the viscosity of the bulk solution remains unchanged.

3.2 Analysis of oleic acid and linoleic acid in peanut oil by CE

The various steps involved in the preparation of a given peanut oil sample derived from a small portion of a single peanut seed, and the subsequent extraction of FFAs from peanut oil are described in Section 2, and summarized in Fig. 1. Okrun (moderate oleic acid with respect to linoleic acid) and ARSOK-R1 (high oleic with respect to linoleic acid) were the primary plant lines under study. In these peanut oil samples, oleic and linoleic acids were readily detected by CE using the partially aqueous electrolyte system described in the above section. Also, a tiny peak of stearic acid (C18:0) could be seen in these samples as peanut oils are known to have about 2–4% of stearic acid [5] (see Fig. 6).

For the quantitative determination of oleic and linoleic acids in peanut oil samples, an IS that shares similar properties with these solutes is the best choice. In this regard, the nonadecanoic acid (C19:0), which is usually absent or present in trace amounts in peanut oils [5], was selected as the IS for establishing the standard calibration curve. The C19:0 being one methyl group longer than the C18:0 migrated right before C18:0 with complete resolution (see Fig. 7). In all its aspects, the C19:0 was therefore a good choice as the IS for analyzing peanut oil samples. Only the determination of the oleic acid and linoleic acid contents of the peanut seed under investigation was of major interest to the present study.

To check the reproducibility of the extraction method employed, the peanut oil obtained from a single seed of each peanut line (Okrun and ARSOK-R1) was split into three equal size oil samples, which were then extracted under the same set of conditions and subsequently ana-

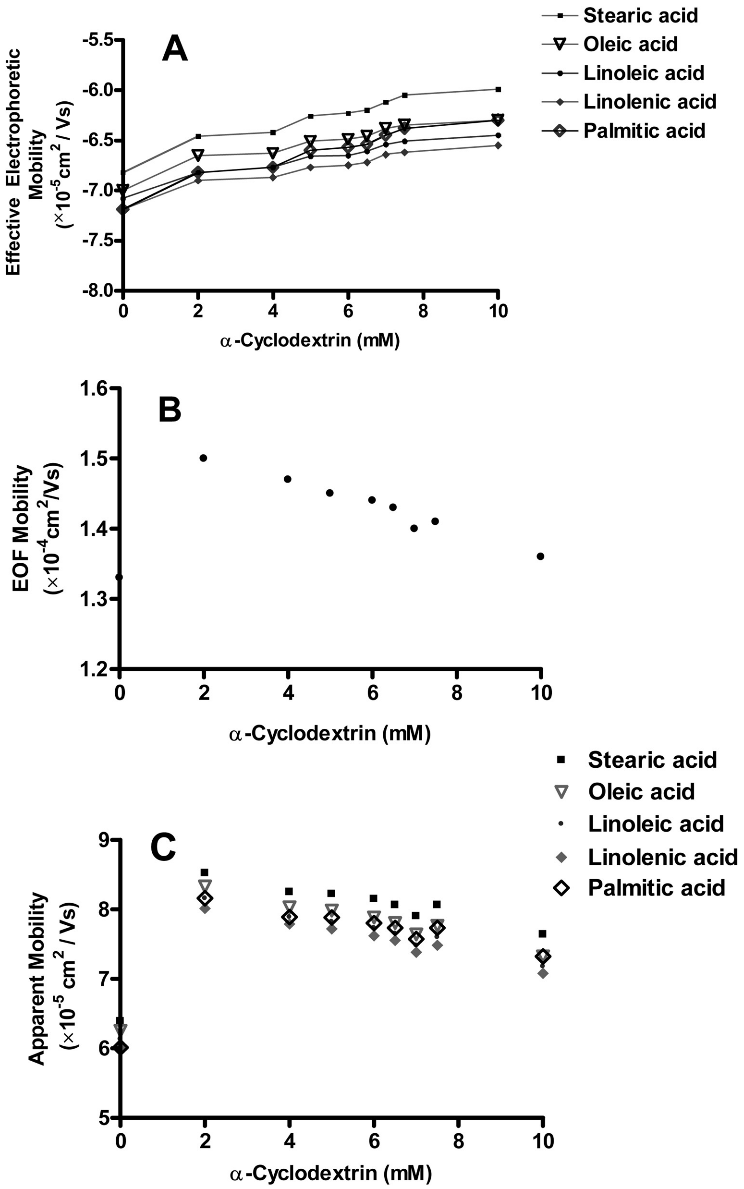

Figure 5. Effect of the concentration of α-CD on (A) effective electrophoretic mobilities, (B) electroosmotic mobility, and (C) apparent mobilities of five standard FFAs in a NMF/dioxane/water (5:3:2 by volume) mixture.

lyzed by CE. The quantitative data in terms of % oleic acid and % linoleic acid are summarized in Table 1. As can be seen in Table 1, the extraction is reproducible as expressed in terms of % RSD, which are 0.79 and 0.44% for oleic acid from ARSOK-R1 and Okrun, respectively, and 1.22 and 4.96% for linoleic acid from ARSOK-R1 and Okrun, respectively.

Eight peanut oil samples derived from eight small portions of eight different peanut seeds each from Okrun and ARSOK-R1 were analyzed. The percent of oleic acid and linoleic acid in these 16 oil samples are given in Table 2. For Okrun, the oleic acid was found in the range of 39.9–59.3% and linoleic acid was in the range of 27–44.2%. For ARSOK-R1, the range for oleic acid was 60.8–85.9% and the linoleic acid was present in the range of 4.8–6.7%.

In all cases, the quantification of oleic acid and linoleic acid was achieved by comparing peak heights of the fatty acids in the sample with that of the standards from the calibration curve in the range of 0.2–1.4 mM. The calibration curves for oleic acid and linoleic acid were linear ($y = 2.232x + 0.1$ for oleic acid and $y = 2.286x$ for linoleic acid) in the concentration range studied with R^2 equal to 0.9985 and 0.9961, respectively. The SDs for the slope and the intercept were 0.09 and 0.06 for oleic acid, respectively, and 0.1 and 0.01 for linoleic acid, respectively.

In all extractions aiming at determining the concentration of a given species in a given matrix, the major concern is the percentage recovery of the particular species from the matrix. In this regard some recovery measurements were undertaken and the results are summar-

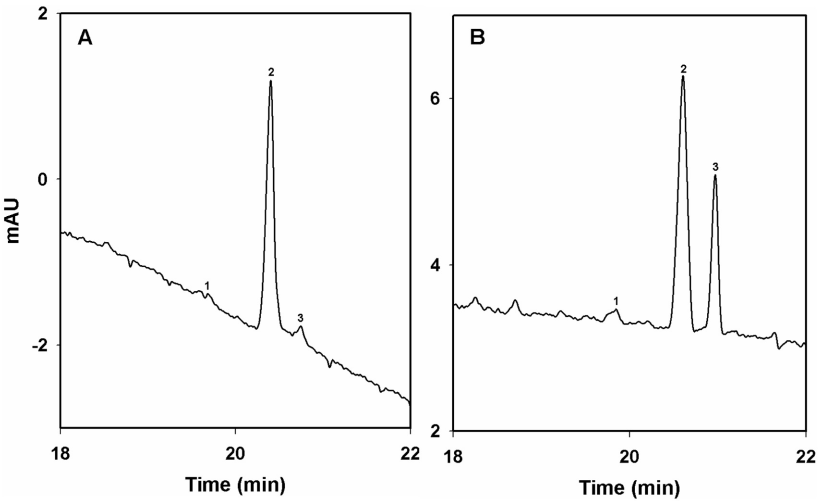

Figure 6. Electropherograms of peanut oil samples from small portions of single peanut seeds of two lines, (A) ARSOK-R1 and (B) Okrun using a partially aqueous electrolyte consisting of 40 mM Tris, 2.5 mM AMP, and 7 mM α-CD in NMF/dioxane/water (5:3:2 by volume) mixture, pH between 8 and 9. Hydrodynamic injection for 3 s at 0.5 psi, applied voltage of 28 kV, and detection at 254 nm. Peak identification: 1, C18:0; 2, C18:1; 3, C18:2.

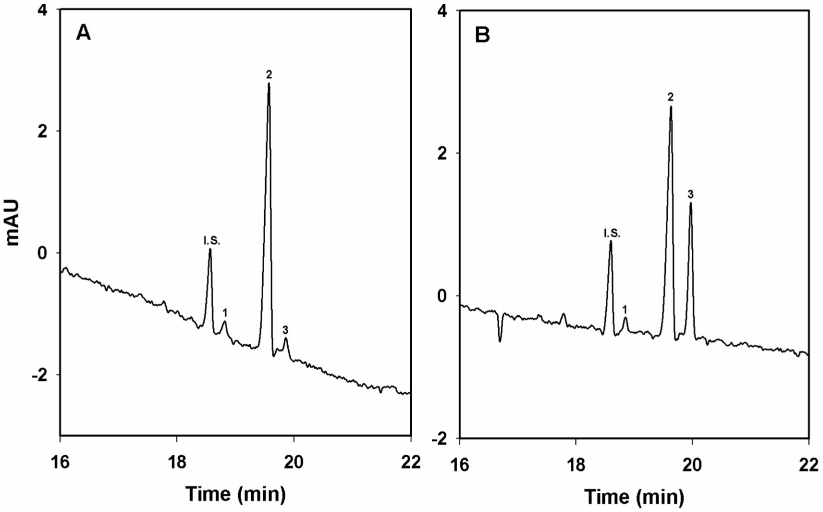

Figure 7. Electropherograms of peanut oil samples from two small portions of two seeds of two peanut lines, (A) ARSOK-R1 and (B) Okrun using a partially aqueous electrolyte consisting of 40 mM Tris, 2.5 mM AMP, and 7 mM α-CD in NMF/dioxane/water (5:3:2 by volume) mixture, pH 8–9. Hydrodynamic injection for 3 s at 0.5 psi, applied voltage of 28 kV, and detection at 254 nm. Peak identification: IS, C19:0; 1, C18:0; 2, C18:1; 3, C18:2.

Table 1. Determination of wt% oleic acid and wt% linoleic acid in peanut oil derived from a single peanut seed of ARSOK-R1 and Okrun peanut lines

Oil portion number	ARSOK-R1		Okrun	
	% Oleic	% Linoleic	% Oleic	% Linoleic
1	85.86	5.25	51.98	36.92
2	87.04	5.31	52.08	33.43
3	85.87	5.38	52.42	35.23
Mean	86.26	5.31	52.16	35.19
SD	0.68	0.07	0.23	1.75
% RSD	0.79	1.22	0.44	4.96

The oil fraction from each seed was divided into three equal portions each of which was subsequently treated and its fatty acids were extracted. Also, values of reproducibility expressed in terms of % relative standard derivation (% RSD) are reported.

Table 2. Determination of wt% oleic acid and wt% linoleic acid in eight samples of peanut oil of ARSOK-R1 and Okrun peanut lines

Peanut seed number	ARSOK-R1		Okrun	
	% Oleic	% Linoleic	% Oleic	% Linoleic
1	60.75	4.87	39.85	27.05
2	62.51	5.5	43.88	29.16
3	73.77	5.04	46.8	37.22
4	73.84	4.94	48.1	38.51
5	76.71	5.37	53.33	39.01
6	78.11	6.39	55.56	42.35
7	79.91	6.65	55.45	42.59
8	83.87	4.83	59.29	44.21

Each oil sample was derived from a small portion of a single peanut seed. The oil fraction from each seed was subsequently treated and its fatty acids were extracted.

ized in Table 3. Here the recovery measurement should be taken using a species that can be added in known amount yet it is not present in the oil but similar in properties to the FFAs. Under these conditions, the best choice was the nonadecanoic acid (C19:0).

Table 3. Percent recovery of nonadecanoic acid (C19:0) from peanut oil during the extraction process described in Section 2

Extraction number	% Recovery	Absolute error	Relative error
1	102.11	5.52	5.71
2	93.44	3.15	3.26
3	94.22	2.37	2.45
Average	96.59	3.68	3.81

In the recovery measurements, a known amount of C19:0 was added to a known amount of peanut oil from a small portion of a single seed so that the final concentration of C19:0 in the final extract and assuming that nothing is lost from it during the extraction process will be the same as that in a standard solution of C19:0. Three extractions of three equally sized oil samples which were derived from a single peanut seed spiked with C19:0 were performed under the same conditions using the steps summarized in Fig. 1. Both the samples extracted from the spiked oil and containing C19:0 and the standard C19:0 solution were run in CE under otherwise identical running conditions. The peak heights of both the samples and the standards were compared thus yielding the percentage recovery listed in Table 3. As can be seen in Table 3, the percentage recovery was ~97% on an average. This percentage recovery is quite impressive given the number of steps involved in the extraction process (see Fig. 1).

4 Conclusions

This investigation has shown the suitability of CE in the analysis of peanut oil derived from a small portion of a single peanut seed for its content of fatty acids. The CE method developed here is based on a partially aqueous electrolyte system and allowed the separation of underivatized FFAs using indirect UV detection. The extraction procedure of FFAs from peanut oil was very reproducible with a ~97% recovery from the seed matrix. Furthermore, the CE method allowed the screening of high oil peanut seeds for breeding programs.

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